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Functional Recovery of Retina After Sodium Iodate Injection in Mice

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ERGs and the azide responses were recorded from mice before and periodically up to 6 weeks after retinal pigment epithelium (RPE) damage by iodate injection to follow the recovery of retinal pigment epithelium and retinal function. At 14 days postinjection, there was a partial recovery of the maximal b-wave amplitude and the azide response but no further recovery was found after 14 days. The retinal sensitivity showed a slow recovery, and at 6 weeks postinjection did not differ from the pre-iodate sensitivity. These findings correlated with histological observations. We concluded that the recovery in ERGs resulted from RPE recovery and the large patchy area of recovered retina functioned normally. © 1997 Elsevier Science Ltd.

Electroretinogram (ERG) Retina Recovery Sodium iodate

INTRODUCTION

The toxic effect of sodium iodate ($NaIO_3$) on the retinal pigment epithelium (RPE) is well known (Noell, 1953, 1954; Grignolo *et al.*, 1966). Earlier studies on the effect of $NaIO_3$ on the RPE used the electroretinogram (ERG) and concentrated on the c-wave, which arises in part from the RPE (Textorius & Weilinder, 1981; Nao-I *et al.*, 1986) to assess the functional changes of the RPE. We have studied the early effects of $NaIO_3$ on the retina using the b-wave of the ERG in mice (Adachi-Usami *et al.*, 1992; Hosoda *et al.*, 1993). In those studies, we found that there was an increase in the b-wave amplitude and a shift of the intensity-response curve towards higher stimulus intensities 24 hr after the intravenous injection of iodate, but 96 hr after injection the waves were greatly attenuated. Histopathologic examination of the retinas at 24 hr showed severe damage in the outer layer of the retina and in the RPE.

In fluorescein angiographic studies, Ringvold *et al.* (1981) reported that there was a breakdown of the RPE barrier within 24 hr after the intravenous injection of iodate, but between 1 and 2 weeks after the injection, no leakage of fluorescein could be seen. Korte *et al.* (1984) reported destruction of the RPE with intravenous injection of $NaIO_3$ cause choriocapillary atrophy, and they also reported that after 1 week they saw no fluorescein leakage. These findings suggested that there was a recovery of RPE function. However, no functional recovery of retina has been reported after $NaIO_3$ injection.

Noell (1952) demonstrated that an intravenous injection of sodium azide in rabbits led to a transient increase in the standing potential of the eye, the azide response. Because the azide response was strongly depressed following an iodate injection which damaged the RPE cells, he concluded that the azide response originated from the RPE. Intracellular recordings from RPE cells showed that the azide response originated from a depolarization of the basal membrane of the RPE and the inner retina did not contribute significantly to the azide response (Linsenmeier & Steinberg, 1987).

In the present study, we followed both the ERG and azide responses (Noell, 1952) for 6 weeks after the $NaIO_3$ injection in order to see electrophysiological recovery, which has never been reported.

MATERIALS AND METHODS

We used 3 week-old BALB/c mice weighing 12 g each. The animals were kept on a 12:12 light:dark schedule. The treatments and procedures on the mice were conducted under intramuscular anesthesia with ketamine (11 mg/kg), xylazine (14 mg/kg) and urethane (500 mg/kg). All the experiments in this study conformed to the Association for Research in Vision and Ophthalmology Resolution on the Use of animal in Research.

$NaIO_3$ was diluted with saline and 40 mg/kg body weight of $NaIO_3$ solution (4 mg/ml) was injected through the caudal vein. For ERG recordings, a cotton-wick electrode was placed on the cornea and was referred to an electrode placed subcutaneously on the nasal bone. The responses were amplified and the preamplifier bandwidth

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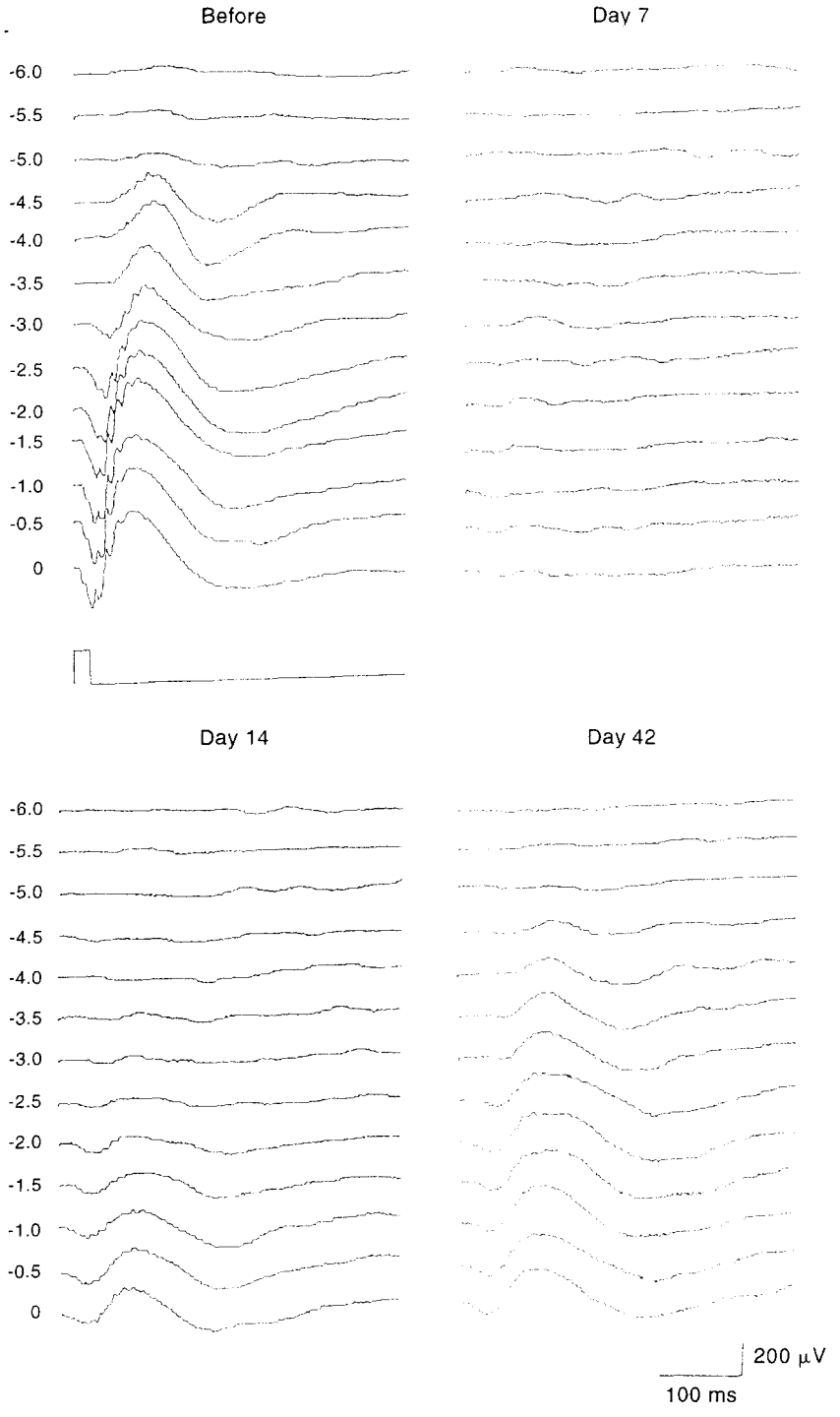

FIGURE 1. Electroretinograms of a BALB/c mouse recorded before and 7, 14 and 42 days after injection of $NaIO_3$. The maximum illuminance (0.0 log intensity) is 140,000 lux.

was set at 1.5 and 100 Hz. The noise level of the recording system was approximately 5 μV, and signals of 10 μV could be easily detected. After the electrodes were in place, the animals were dark-adapted for 30 min. Single flash ERGs were recorded.

The stimulus light for eliciting ERGs was obtained from a 150 W quartz–halogen light bulb. The light was collected and focused onto a 3 mm diameter fiber optic bundle. The tip of the bundle was placed 0.5 cm from the cornea. The illuminance of the unattenuated stimulus on the surface of the cornea was 140,000 lux, which is 0 log intensity. Neutral density filters (NDFs) were used to reduce the stimulus intensity. The duration of the stimulus was controlled at 20 msec by an electromagnetic shutter.

The b-wave amplitude was measured from the bottom of the a-wave to the top of the b-wave, and the b-wave amplitude vs log stimulus intensity curve was studied. Responses were recorded beginning with 6.0 log neutral density, which was close to the threshold for a b-wave

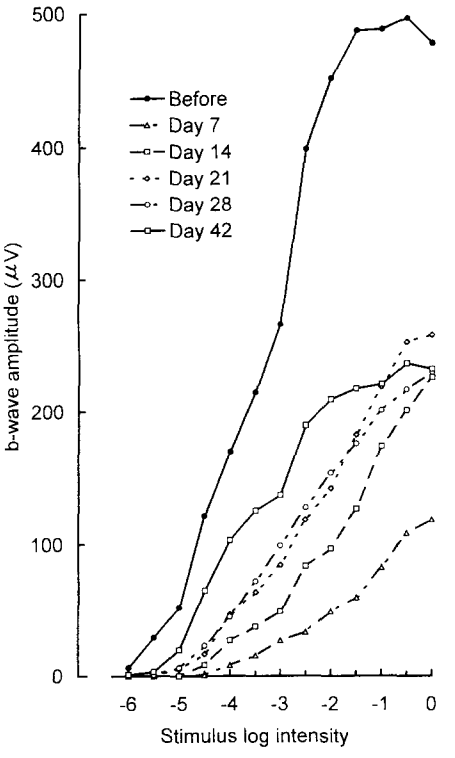

FIGURE 2. The mean b-wave amplitude ($n = 12$) vs log stimulus intensity before and 7, 14, 21, 28, and 42 days after injection of $NaIO_3$.

from the control eyes. The stimulus intensity was increased by 0.5 log steps to the full intensity stimulus with a 1 min interval between stimuli. In each mouse, ERGs were recorded before and 1, 4, 7, 14, 21, 28 and 42 days after 40 mg/kg of $NaIO_3$ was injected through the caudal vein.

To record the azide response, electrodes were placed as for the ERG recording and 0.1 ml of sodium azide (NaN_3) solution (1.0 mg/ml, diluted with saline) was injected manually through the caudal vein within 1 sec. The responses were amplified with the bandwidth of the preamplifier set between 0.08 and 30 Hz. The amplitude of azide response was measured from the baseline to the peak of the positive wave that peaked about 4 sec after the injection of NaN_3 solution. In each of the 12 animals injected with iodate and the five saline-injected controls, the azide responses were recorded before and 1, 7, 14, 21, 28 and 42 days after the injection of $NaIO_3$.

For histopathological studies, 10 additional mice were used. The animals were perfused through the heart with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer under deep anesthesia. The enucleated eyes were kept in the same solution for 30 min; then the anterior segments were removed. The eyecups were further dissected into small pieces. These tissues were embedded in paraffin. Six-micron sections were cut and stained with hematoxylin and eosin for light microscopic studies.

TABLE 1. The mean and standard error of V_{max} and I_{50}

Days after injection	V_{max} (μV)	I_{50} (log intensity)
Pre	512 ± 37	3.47 ± 0.08
1	1107 ± 91	2.93 ± 0.10
4	219 ± 24	1.82 ± 0.15
7	138 ± 14	1.54 ± 0.22
14	261 ± 18	1.74 ± 0.30
21	289 ± 20	2.12 ± 0.26
28	263 ± 18	2.32 ± 0.29
42	254 ± 23	3.00 ± 0.25

RESULTS

ERG studies

The changes induced in the ERGs of a mouse injected with $NaIO_3$ are shown in Fig. 1. On day 7 postinjection, only very small ERGs were recorded with the two highest stimulus intensities. On day 14 postinjection, there was an increase in the amplitude of the ERGs and both a- and b-waves were recorded at the higher intensity levels. On day 42 postinjection, there was a further increase in ERG amplitude for dimmer flashes.

From ERGs such as these, the b-wave amplitude was measured for the 12 mice, and the mean intensity–response curves for the 12 animals are shown in Fig. 2. Before $NaIO_3$ injection, a small b-wave was elicited with the −6.0 log stimuli, and the amplitude increased until 1.5 log units attenuation, where it saturated.

As we previously reported (Adachi-Usami *et al.*, 1992; Hosoda *et al.*, 1993), the b-wave amplitudes on day 1 postinjection were significantly larger than controls, especially at the higher stimulus intensities. However, on days 4 and 7 postinjection, b-wave amplitudes were attenuated at all stimulus intensities. By day 14, the b-wave amplitudes were larger than on day 7, and on days 21, 28 and 42, the b-wave amplitudes continued to increase at the lower stimulus intensities even though the maximum b-wave amplitude remained similar to that on day 14 postinjection.

A Naka–Rushton analysis was done to evaluate V_{max} (maximum b-wave amplitude) and I_{50} (stimulus intensity required to elicit a b-wave of one-half of the V_{max}) for the intensity–response curve of each animal (Table 1, Fig. 3,

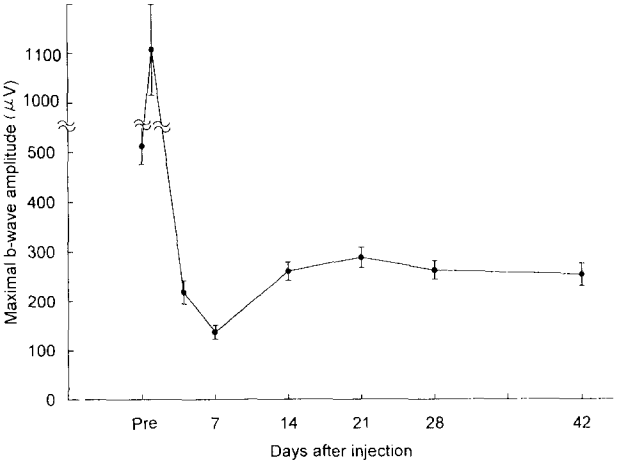

FIGURE 3. The mean and standard error of maximal b-wave amplitude (V_{max}) before and 1, 4, 7, 14, 21, 28, and 42 days after injection of $NaIO_3$ ($n = 12$).

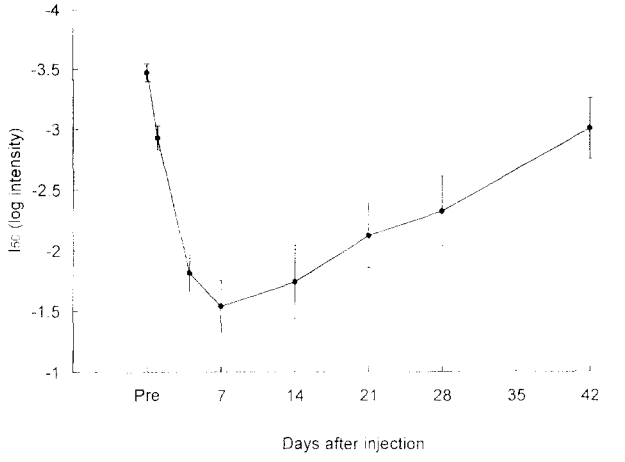

FIGURE 4. The mean and standard error of the stimulus intensity required to elicit a b-wave of one-half of the maximal amplitude (I_{50}) before and 1, 4, 7, 14, 21, 28, and 42 days after injection of $NaIO_3$ ($n = 12$).

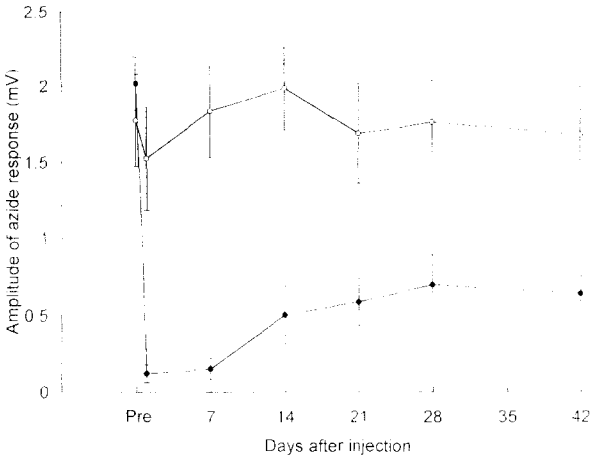

FIGURE 6. The mean and standard error of azide response of control mice ($n = 5$, open circle) and $NaIO_3$-injected mice ($n = 12$, closed circle) before and 1, 7, 14, 21, 28, and 42 days after injection.

Fig. 4). V_{max} 7 days after iodate administration was reduced significantly ($P < 0.01$) compared with controls. On day 14 postinjection, V_{max} had recovered and was significantly ($P < 0.01$) larger than V_{max} on day 7. Thereafter, V_{max} did not increase significantly, although I_{50} sensitivity continued to increase up to day 42.

Azide response studies

The azide response was first studied in control mice (Fig. 5). Intravenous injection of NaN_3 elicited a large corneal-positive wave of approximate 2 mV amplitude and peaked at about 4 sec after the beginning of the injection. Repetitive injection of azide did not change the amplitude of azide response in the control mice.

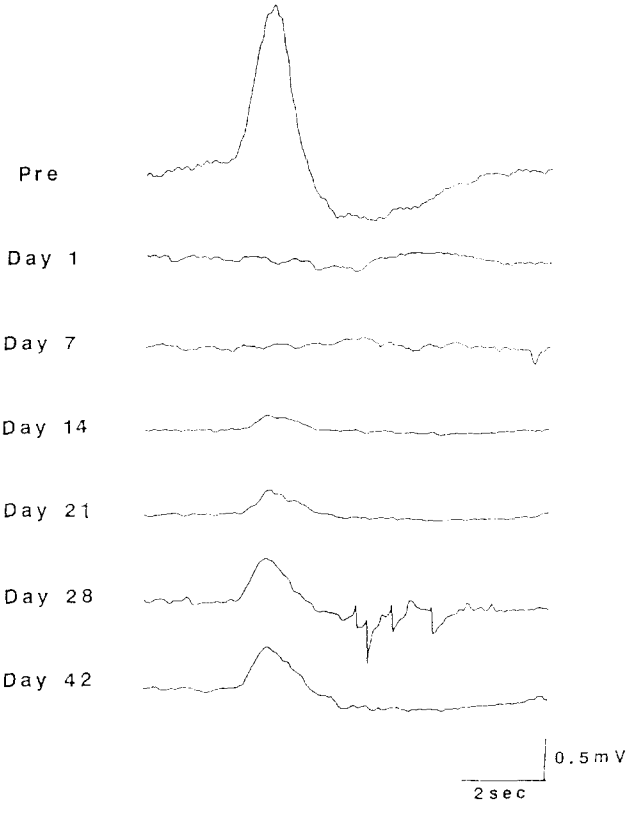

FIGURE 5. The wave of azide response of a BALB/c mouse recorded before and 1, 7, 14, 21, 28, and 42 days after injection of $NaIO_3$.

The azide responses recorded from one mouse before and 1, 7, 14, 21, 28 and 42 days after injection of $NaIO_3$ are shown in Fig. 5. On days 1 and 7 after the iodate injection, no azide response could be elicited. On day 14 postinjection, a small azide response was recorded, and with increasing postinjection days, the azide response became larger.

The time course of the changes in the azide response in the iodate-injected mice ($n = 12$) and control mice ($n = 5$) are shown in Fig. 6. From day 1 to day 7 postinjection, the amplitude of the azide response decreased significantly but then showed some recovery, with the response significantly larger on day 14 than on day 7 ($P < 0.05$). Thereafter, the azide response, as V_{max}, did not increase significantly but remained at about 25% of the pre-iodate level.

Histopathological study

Histological examinations of the retina were conducted in control and $NaIO_3$ injected mice 14 and 42 days after injection (Fig. 7). In control retina, long outer segments of photoreceptor could be seen [Fig. 7(A, B)]. Fourteen days after iodate injection, outer segments of photoreceptor had almost disappeared and the RPE was replaced by a layer of a few flat, modified RPE cells [Fig. 7(C, D)]. After 42 days, the RPE resembled its appearance on day 14. The number of photoreceptor cells was lower than in control retinas such that the number of outer nuclear layer cells decreased by about 70% in the central retina, and 50% in periphery. In some regions, the photoreceptors were absent and cells of the inner nuclear layer were apposed to Bruch's membrane [Fig. 7(E, F)]. In other parts, RPE cells were still present and normal retinal structure was preserved, and outer segments of photoreceptor existed in these areas. The morphological retinal changes were more extensive in the central retina

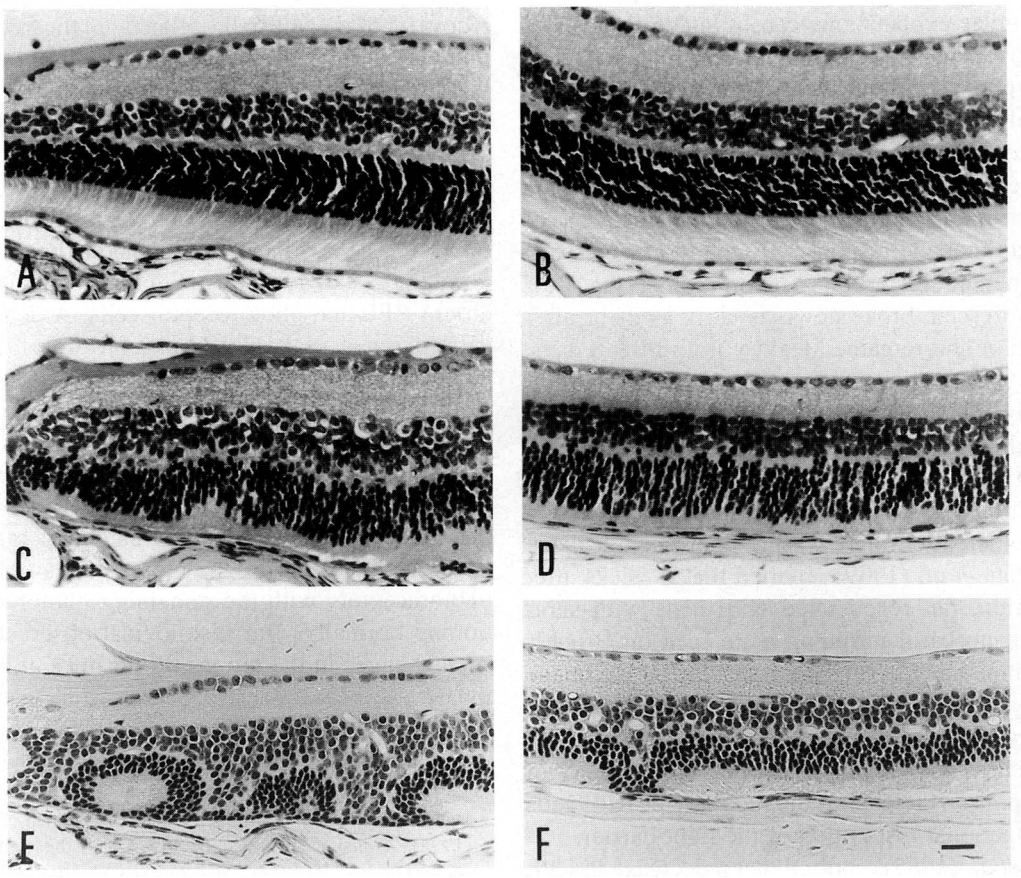

FIGURE 7. Light micrograph of central part (A, C, E) and peripheral part (B, D, F) of the retina of control mouse (A, B), 14 days (C, D), and 42 days (E, F) after injection of $NaIO_3$ (scale bar = 20 μm).

than in the periphery. The cells of the inner retina, on the other hand, were fairly well preserved.

DISCUSSION

In this study, we have demonstrated that the early depression of the b-wave following iodate injection is followed by a slow increase. This recovery of ERG has not been described previously. The toxic effects of $NaIO_3$ have been attributed to the inhibition or suppression of several enzymes in the RPE cells (Hayasaka *et al.*, 1988), and also to the breakdown of the blood–retinal barrier (Kitano *et al.*, 1988). These functional changes in the RPE cells would then account for the depression of the c-wave and the azide response.

In earlier iodate studies (Textorius & Weilinder, 1981; Nao-I *et al.*, 1986), the c-wave was used to assess the functional state of the RPE. The depression of the c-wave and azide response following iodate injection was attributed to the alteration of the RPE cells as severe morphological damage of the RPE was found to accompany these physiological changes. However, it should be remembered that the c-wave arises from the summation of potential changes, not only in the RPE but also from the inner retina, namely, the slow PIII. In addition, because the c-wave results from the decrease in K^+ in the subretinal space due to photoreceptor activity, damage to the photoreceptors can also result in smaller c-waves. Thus, when the mechanism for the depression of the c-wave is considered, these other sources must also be examined.

The azide response has been shown to result from the depolarization of the basal membrane of the RPE, and the inner retina does not contribute significantly to the azide response (Linsenmeier & Steinberg, 1987). In addition, the azide response does not require photoreceptor activation. Thus, the azide response provides an independent assay of the RPE cells.

There was a good correlation between the changes in the azide response and maximum b-wave amplitude (V_{max}). Initially, there was a decrease in the both the azide response and V_{max} following iodate injection which was followed by a recovery up to 14 days postinjection. Thereafter, there was no further recovery of either response. I_{50}, on the other hand, showed a much slower rate of recovery and continued to improve until day 42. At this time the sensitivity did not differ from the pre-iodate level. Thus, at 6 weeks, V_{max} and the azide response were significantly depressed even though I_{50} had mostly recovered.

It was interesting to note that intravenous injection of iodate resulted in patchy degeneration of the RPE at 42 days after the injection. Marmor & Dalal (1993) also reported patchy, irregular retinal and RPE damage after pressure-induced ischemia, and they speculated this might result from variations in local cellular mechanism, local vascular anatomy, or in local cellular recovery

potential. Similar explanations can be made to account for the patchy degeneration after $NaIO_3$ injection. The fact that morphologically the central retina was damaged more severely than the peripheral retina may provide some clues to this patchy change.

There have been several histological and angiographic studies which might explain the functional recovery of the ERG and the azide response. Ringvold *et al.* (1981) who experimented on rabbits, reported that the RPE barrier to fluorescein broke down as early as 24 hr after $NaIO_3$ injection and remained leaky for up to 7–8 days. The barrier was then restored in another 1–2 days. Histologically, by day 9, a continuous cell layer with many intercellular contacts replaced the RPE and acted as a functional tight barrier. Korte *et al.* (1984) also reported a RPE barrier breakdown up to 1 week after the injection of iodate but thereafter, there was no fluorescein leakage in rabbit. Ogata *et al.* (1989) reported that 2 weeks after $NaIO_3$ injection, flat regenerated RPE cells with short microvilli but no basal infolding were seen on Bruch's membrane in the rat. The time course of the RPE damage and recovery in these reports were very similar to the time course of recovery of V_{max} and the azide response in our experiment. This would suggest that the recovery of the azide response is related to the recovery of RPE cells and/or the functional restoration of the tight barrier.

Steinberg *et al.* (1985) reported that a decrease in RPE resistance led to a increase in b-wave amplitude. From the results of our electrophysiological and histopathological observations, we suggest that the iodate injection induced both large areas of RPE damage with a breakdown in the RPE–blood barrier and photoreceptor damage, and that the damage in photoreceptors caused the decrease in b-wave. During the 2 weeks following the injection, there is regeneration and recovery of the RPE which would then support the surviving photoreceptor cells, leading to their gradual recovery. Two weeks postinjection, the recovery of the RPE is complete but the functional recovery of photoreceptors continues until the sixth week. In the areas with complete RPE loss, there is no recovery of the photoreceptors.

The results of this study have important relevance to the effect of retinal pathology on the Naka–Rushton parameters. Our observations showed that V_{max} recovered up to 2 weeks after the iodate injection but did not show any further recovery. I_{50}, however, showed slight recovery at 2 weeks but continued to recover until 6 weeks, at which time the sensitivity was not significantly different from that preceding the injection. Histologically at this time, there were large areas of the retina devoid of RPE cells and photoreceptors and other areas where RPE cells and photoreceptors were present. The inner layers of the retina were usually well preserved after $NaIO_3$ injection. Thus, the changes in the Naka–Rushton parameters must be related to changes in the photoreceptors and the RPE. The recovery of the RPE as assessed by the azide response was complete at 2 weeks and thus cannot account for the slow recovery in I_{50} between weeks 2 and 6. This then indicates that the slow recovery of I_{50} must be related to the recovery of the photoreceptors. Taken together, our observations indicate that the decrease of V_{max} results from the large patches of retina with missing photoreceptors, and the decreased azide response is due to the patches of retina where RPE cells are absent. The slow recovery of I_{50} results from the slow recovery of the photoreceptors in the areas of recovered RPE cells. Thus, retinas with reduced V_{max} but normal I_{50} can result from retinas with large areas of absent RPE and photoreceptor cells. Arden *et al.* (1983) carried out a Naka–Rushton analysis of the ERGs of heterozygous patients with X-linked RP and found that the V_{max} was significantly reduced but I_{50} did not differ from normal patients. These are the changes in the Naka–Rushton parameters in our mice at 6 weeks post-iodate. It is noteworthy that Arden *et al.* (1983) suggested that these changes in the Naka–Rushton parameters could have resulted from the loss of large patches of photoreceptors with the remaining photoreceptors functioning normally. Our histological observations showed such changes in the retinas of our mice at 6 weeks post-iodate.

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